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REMARKS/ARGUMENTS

Claims 22, 26, 30-32, 34-37, 56, 70-76, 81-88 and 90-95 are active in this application.

Support for the amendment to Claim 26 is found in Claims 28 and 29 as well as in the specification as originally filed. The remaining amendments are for clarity and to more clearly set forth the claimed invention. No new matter is added. Applicant requests that the Examiner reconsider rejoining Claims 74 and 75. Claims 74 and 75 are dependent on Claim 56 which has already been rejoined to the elected subject matter.

Applicant thanks the Examiner for indicating that Claim 22 is allowed and the subject matter of Claim 69 is allowable. Applicant also thanks the Examiner for the discussion granted to the Applicant's undersigned representative on May 25, 2004. During this meeting, amendments to the claims to address the outstanding §112 rejections were discussed. In particular, it was discussed to define the α and β subunit by nucleotide sequences and activity, which are reflected in the claims submitted herein.

The rejection of Claims 26 and 28-37 under 35 U.S.C. § 112, first paragraph is respectfully traversed.

As noted above, Claim 26 has been amended to define the phosphotransferase enzyme as being encoded by SEQ ID NO:3 or sequences that hybridize to SEQ ID NO:3. In addition, those sequences which hybridize to SEQ ID NO:3 when combined with an α or β subunit encoded by SEQ ID NO:3 has phosphotransferase activity. Accordingly, Applicant requests withdrawal of this ground of rejection.

The rejection of Claims 56, 57 and 70-73 under 35 U.S.C. § 112, first paragraph is also respectfully traversed.

Claim 56 as amended herein is directed to a method of phosphorylating a lysosomal hydrolase protein which comprises an asparagine-linked oligosaccharide with a high mannose structure. Accordingly, withdrawal of this ground of rejection is requested.

The rejection of Claims 35 and 70 under 35 U.S.C. § 112, second paragraph is respectfully traversed.

During the discussion with the Examiner, the Examiner requested clarification of the recognition sequences for the proteolytic enzymes. In particular, it was indicated that pointing out the consensus cleavage sites of the four proteolytic enzymes recited in the claims would address this ground of rejection. Accordingly, Applicant attaches herewith printouts from the New England BioLabs® on-line catalogue for Enterokinase, Factor Xa, Furin, and Genenase I:

1. the recognition sequence for enterokinase is D-D-D-D-K,
2. the recognition sequence for Factor Xa is I-E/D-G-R,
3. the recognition sequence for Furin is R-X-X-R, and
4. the recognition sequence for Genenase is H-Y or Y-H.

A comparison of these substrate recognition sites to the sequences presented in Figure 5 and SEQ ID NOS:22-25 reveals that these substrate recognition sites are present in SEQ ID NO:22-25.

The rejection as it stands to Claim 70 is addressed by amendment.

In view of the foregoing, Applicant requests withdrawal of the rejection under 35 U.S.C. § 112, second paragraph.

The objection of Claims 26, 28-37 and Claim 70 have been addressed by amendment.

Applicants also request a returned signed copy of the Information Disclosure Statement filed on December 22, 2003. For reference, a copy of that Information Disclosure Statement is attached.

Finally, Applicant requests allowance of this application. Early notice of such allowance is requested.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



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Enterokinase, light chain



| Catalog # | Size | Concentration | Price | Qty | |
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| P8070S | 0.063 µg | 2 µg/ml | \$84.00 | <input type="text" value="1"/> | <input type="button" value="ADD TO CART"/> |
| P8070L | 0.032 µg | 2 µg/ml | \$336.00 | <input type="text" value="1"/> | <input type="button" value="ADD TO CART"/> |

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Substrate Specificity:

Asp-Asp-Asp-Asp-Lys[†]

Description:

Enterokinase is a specific protease that cleaves after lysine at its cleavage site Asp-Asp-Asp-Asp-Lys. It will sometimes cleave at other basic residues, depending on the conformation of the protein substrate. Enterokinase will not cleave at site followed by proline.

Source:

This preparation is purified from *K. lactis* containing a clone of the light chain of the bovine enterokinase gene (1,2).

Enzyme Properties

Specificity: Enterokinase is a specific protease that cleaves after lysine at its cleavage site Asp-Asp-Asp-Asp-Lys[†].

Molecular Weight:

Theoretical: 26.3 kDa and Apparent: 31 kDa

Reaction & Storage Conditions

Unit Definition:

0.00016 µg of Enterokinase will cleave 25 µg of test substrate to 95% completion in a total reaction volume of 50 µl in 16 hours or less at 23°C in 20 mM Tris-HCl (pH 8.0 @ 25°C) with 50 mM NaCl and 2 mM CaCl₂.

Concentration:

2 µg/ml

Storage Conditions:

20 mM Tris-HCl
200 mM NaCl
2 mM CaCl₂
50% glycerol
pH 7.2 @ 4°C

Storage Temperature:

-20°C

References

1. Collins-Racie, L.A. et al (1995) *Biotechnology*, 13, 982-987.
2. Taron, C. and Colussi, P., unpublished observations.

Companion Products

Factor Xa
Furin
Genenase I
Proteinase K

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Licensing Text:

This product is sold under patent license from Genetics Institute, Inc. for research use only. Licenses for commercial manufacture or use may be obtained directly from Genetics Institute, Inc., 87 Cambridgepark Drive, Cambridge, MA 02140.

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Factor Xa

| Catalog # | Size | Concentration | Price | Qty | |
|-----------|--------|---------------|----------|--------------------------------|-----------------------------|
| P8010S | 50 µg | 1 mg/ml | \$53.00 | <input type="text" value="1"/> | ADD TO CART |
| P8010L | 250 µg | 1 mg/ml | \$212.00 | <input type="text" value="1"/> | ADD TO CART |

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Substrate Specificity:

Ile-Glu/Asp-Gly-Arg▼

Description:

Factor Xa cleaves after the arginine residue in its preferred cleavage site Ile-(Glu or Asp)-Gly-Arg. It will sometimes cleave at other basic residues, depending on the conformation of the protein substrate (1,2,3). The most common secondary site, among those that have been sequenced, is Gly-Arg. There seems to be a correlation between proteins that are unstable in *E.coli* and those that are cleaved by Factor Xa at secondary sites; this may indicate that these proteins are in a partially unfolded state (Walker, I., Riggs, P., unpublished observations). Factor Xa will not cleave a site followed by proline or arginine.

Source:

Factor Xa protease is purified from bovine plasma and activated by treatment with the activating enzyme from Russell's viper venom.

Enzyme Properties

Deactivation: Dansyl-glu-gly-arg-chloromethyl ketone (CALBIOCHEM, #251700) will irreversibly inactivate Factor Xa by covalent attachment at the active site. In a reaction containing 20 µg/ml Factor Xa, 2 µM dansyl-glu-gly-arg-chloromethyl ketone will inactivate >95% of the Factor Xa in 1 minute at room temperature.

Molecular Weight:

Theoretical: 43 kDa and Apparent: 50 kDa

Reaction & Storage Conditions**Unit Definition:**

1 µg of Factor Xa will cleave 50 µg of MBP fusion protein test substrate, MBP-ΔSal to 95% completion in a total reaction volume of 50 µl in 6 hours or less at 23°C in 20 mM Tris-HCl (pH 8.0 @ 25°C) with 100 mM NaCl and 2 mM CaCl₂.

Concentration:

1 mg/ml

Storage Conditions:

20 mM HEPES

500 mM NaCl
2 mM CaCl₂
50% glycerol
pH 8.0 @ 25°C

Storage Temperature:
-20°C

Notes

General notes:

1. The test substrate MBP-ΔSal is maltose-binding protein fused to a truncated form of paramyosin, with the amino acids Ile-Glu-Gly-Arg at the fusion joint. Greater than 95% of the fusion protein is cleaved in 6 hours or less.

References

1. Nagai, K. et al. (1984) *Proc. Natl. Acad. Sci. USA*, 82, 7252-7255.
2. Quinlan, R.A. et al. (1989) *J. Cell Sci.*, 93, 71-83.
3. Eaton, D. et al. (1986) *Biochemistry*, 25, 505-512.

Companion Products

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Furin

| Catalog # | Size | Concentration | Price | Qty | |
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| P8077S | 50 units | 2,000 units/ml | \$105.00 | <input type="text" value="1"/> | ADD TO CART |
| P8077L | 250 units | 2,000 units/ml | \$420.00 | <input type="text" value="1"/> | ADD TO CART |

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Substrate Specificity:

Arg-X-X-Arg ▾

Description:

Furin is a ubiquitous subtilisin-like proprotein convertase. It is the major processing enzyme of the secretory pathway and is localized in the trans-golgi network (1,2). Substrates of Furin include blood clotting factors, serum proteins and growth factor receptors such as the insulin-like growth factor receptor (3). The minimal cleavage site is Arg-X-X-Arg'. However, the enzyme prefers the site Arg-X-(Lys/Arg)-Arg'. An additional arginine at the P6 position appears to enhance cleavage (4). Furin is inhibited by EGTA, a1- Antitrypsin Portland (5) and polyarginine compounds (6).

Source:

Isolated from *Spodoptera frugiperda* (Sf9) cells infected with recombinant baculovirus carrying truncated human furin (kindly provided by R. Fuller) (3).

Enzyme Properties

Specificity: The minimal cleavage site is Arg-X-X-Arg. However, the enzyme prefers the site Arg-X-(Lys/Arg)-Arg.

Molecular Weight:

Theoretical: 52.7 kDa and Apparent: 57 kDa

Reaction & Storage Conditions

Unit Definition:

One unit is defined as the amount of Furin that will release 1 pmol of AMC from the fluorogenic peptide BOC-RVRR-AMC (Bachem #I-1645) in a total reaction volume of 100 µl in one minute (1 pmol of AMC/min) at 30°C in 100 mM HEPES (pH 7.5 @ 25°C) with 0.5% Triton X-100, 1 mM CaCl₂, 1 mM 2-mercaptoethanol and 100 µM BOC-RVRR-AMC.

Concentration:

2,000 units/ml

Storage Conditions:

10 mM MES

1 mM CaCl₂

10% glycerol

pH 7.0 @ 25°C

Storage Temperature:
-70°C

Notes

Usage notes:

1. Both Furin and *Onchocerca volvulus* Blisterase (NEB #P5204) will cleave peptide substrates with the sequence, Arg-X- (Lys/Arg)-Arg. However, the ability of either enzyme to cleave a particular protein substrate depends on its tertiary structure as well as on the amino acids immediately surrounding the cleavage site (7).

References

1. van den Ouwehand, A.M.W. et al. (1990) *Nucl. Acids Res.*, 18, 664.
2. Steiner, D.F. (1998) *Curr. Opin. Chem. Biol.*, 2, 31-39.
3. Bravo, D.A. et al. (1994) *J. Biol. Chem.*, 269, 25830-25837.
4. Krysan, D.J. et al. (1999) *J. Biol. Chem.*, 274, 23229-23234.
5. Jean, F. et al. (1998) *Proc. Natl. Acad. Sci. USA*, 95, 7293-7298.
6. Cameron, A. et al. (2000) *J. Biol. Chem.*, 275, 36741-36749.
7. Poole, C.B. et al. (2003) *J. Biol. Chem.*, 278, 36183-36190.

Companion Products

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Genenase I

| Catalog # | Size | Concentration | Price | Qty | |
|-----------|--------|---------------|----------|--------------------------------|-----------------------------|
| P8075S | 50 µg | 1 µg/ml | \$53.00 | <input type="text" value="1"/> | ADD TO CART |
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Substrate Specificity:

His-Tyr[▼]

Tyr[▼]-His

Description:

Genenase™ I is a variant of subtilisin BPN' that has been engineered to have increased specificity by substituting amino acids in its active site (1,2). When designing fusion proteins for cleavage with Genenase I, we recommend the site Pro-Gly-Ala-Ala-His-Tyr. Genenase I will cleave at other histidine residues depending on the surrounding amino acids and the 3-dimensional conformation of the protein. Genenase I cleaves His-Tyr-Glu and His-Tyr-Asp slowly, but will not cleave His-Tyr-Pro or His-Tyr-Ile (2).

Source:

Isolated from a *Bacillus subtilis* strain carrying the protease gene derived from *Bacillus amyloliquefaciens*.

Enzyme Properties

Specificity: Genenase I cleaves His-Tyr-Glu and His-Tyr-Asp slowly, but will not cleave His-Tyr-Pro or His-Tyr-Ile (2).

Molecular Weight:

Theoretical: 27.4 kDa and Apparent: 28 kDa

Reaction & Storage Conditions

Unit Definition:

0.5 µg of Genenase I will cleave 50 µg of test substrate to 95% completion in a total reaction volume of 50 µl in 8 hours or less at 23°C in 20 mM Tris-HCl (pH 8.0 @ 25°C) with 200 mM NaCl.

Concentration:

1 µg/ml

Storage Conditions:

20 mM Tris-HCl
200 mM NaCl
2 mM CaCl₂

1 mM dithiothreitol
50% glycerol
pH 7.2 @ 4°C

Storage Temperature:
-20°C

Notes

General notes:

1. The rate of Genenase™ I cleavage has been shown to depend on the amino acid following the cleavage site (position P1')(3). Rates of cleavage when P1' is Asp or Glu are slowest, 40-fold slower than when P1' is Arg or Cys. The rate when P1'= Glu or Asp is enhanced 10- and 14-fold, respectively, by including 2 M KCl in the reaction.

References

1. Carter, P. and Wells, J.A. (1987) *Science*, 237, 394-399.
2. Carter, P. et al. (1991) *Biochemistry*, 30, 6142-6148.
3. Carter, P. et al. (1989) *Proteins: Structure, Function, and Genetics*, 6, 240-248.

Companion Products

Enterokinase, light chain
Factor Xa
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New England Biolabs, Inc.: U.S. 08/090,902
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OSMM&N File No. 20351SUS77

Serial No. 10/023,888

In the matter of the Application of: William M. CANFIELD
For: SOLUBLE GLCNAC PHOSPHOTRANSFERASE

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SERIAL NO: 10/023,888

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SIR:

Applicant(s) wish to disclose the following information.

REFERENCES

- The applicant(s) wish to make of record the references listed on the attached International Search Reports and on Form PTO-1449. Copies of the listed references are attached, where required, as are either statements of relevancy or any readily available English translations of pertinent portions of any non-English language references.
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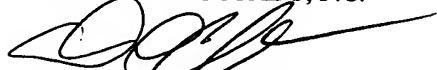
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Respectfully submitted,

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| OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, etc.) | | | | | | | |
| | AS | Sasaki, K., et al., "Expression cloning of a novel alpha 2,3-sialyltransferase using lectin resistance selection. J. Biol. Chem., October 1993, Vol. 268, No. 30, pages 22782-22787 | | | | | |
| | AT | Michel, B., et al., "Selection of an expression host for human glucocerebrosidase: importance of host cell glycosylation. UCLA Symposia on Molecular and Cellular Biology, 1990, Vol. 111 (Glycobiology), pages 159-172. | | | | | |
| | AU | Stanley, P., et al., "Selection and characterization of eight phenotypically distinct lines of lectin-resistant chinese hamster ovary cells. Cell, October 1975, Vol. 6, No. 2, pages 121-128. | | | | | |
| | AV | Zhao, K.W., et al., "Purification and Characterization of Human Lymphoblast N-acetylglucosamine-1-phosphotransferase. Glycobiol. 1992, Vol. 2, No. 2, pages 119-125 | | | | <input type="checkbox"/> Additional References sheet(s) attached | |
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| | BW | Mullis, K.G., Purification and Kinetic Parameters of Bovine Liver N-acetylglucosamine-1-phosphodiester-alpha-N-acetylglucosaminidase. J. Biol. Chem., January 1994, Vol. 269, No. 3, pages 1718-1726. | | | | | |
| | BX | Do, H. et al., "Human Mannose 6-Phosphate-uncovering Enzyme is Synthesized as a Proenzyme that is Activated by the Endoprotease Furin. August 2002, J. Biol. Chem., Vol. 277, No. 33, pages 29737-29744. | | | | | |
| | BY | Lee, W.S., "Multiple Signals Regulate Trafficking of the Mannose 6-Phosphate-uncovering Enzyme", February 2002, Vol. 277, No. 5, pages 3544-3551 | | | | | |
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